Crude Protein by Combustion Method

1.0 Scope

This method applies to the determination of crude protein in solid animal feeds containing 0.2 to 20% nitrogen (1.25 to 125% protein equivalent).

2.0 Summary

Sample is combusted at high temperature in pure oxygen to free nitrogen. Liberated nitrogen gas is isolated from other combustion products and nitrogen oxides are reduced to molecular nitrogen. Total nitrogen is measured by a thermal conductivity detector and expressed as equivalent % protein by applying the appropriate conversion factor.

3.0 Apparatus and Equipment

3.1 Any instrument or device designed to measure nitrogen by combustion may be used provided it is equipped with the following and meets the following requirements:

3.1.1 A furnace capable of maintaining a minimum operating temperature of 950ºC for pyrolysis of the sample in pure (99.9%) oxygen. The manufacturer's design may require a higher temperature.

3.1.2 A system of isolating the liberated nitrogen gas from other combustion products for subsequent measurement by a thermal conductivity detector. A mechanism for converting nitrogen oxides to molecular nitrogen or a means of measuring nitrogen as nitrogen dioxide may be required and included in the design.

3.1.3 A mechanism for interpreting detector response as w/w percent nitrogen in the sample. This may include such features as calibration on standard material, blank determination and barometric pressure compensation. Any required instrument calibration must be based on the theoretical percent of nitrogen in a pure primary standard organic material such as EDTA.

3.1.4 The accuracy requirement is tested by making 10 successive determi-

nations of nitrogen in nicotinic acid and 10 successive determinations in lysine HCl. The means of the determinations must be within $\pm$ 0.15 of the respective theoretical values, with standard deviations $\leq$ 0.15. Standard tryptophan may be substituted for lysine HCl.

3.2 Printer.

3.3 Balance - Accurate to 0.1 mg.

3.4 Barometer - Mercury type, readable to 1 mm.

3.5 Gases:

3.5.1 Carrier gas - 99.99% Helium 40 $\pm$ 4 PSI.

3.5.2 Combustion gas - 99.99% Oxygen 40 $\pm$ 4 PSI.

3.5.3 Pneumatic gas - Compressed air, oil and water free 40 $\pm$ 4 PSI

3.6 Tin Weighing Capsules.

3.7 Glass Wool.

4.0 Chemicals and Standard

4.1 Aluminum Oxide Pellets.

4.2 Anhydrous Magnesium Perchlorate.

4.3 Sodium Hydroxide on an Inert Base.

4.4 Copper Sticks.

4.5 Copper Turnings.

4.6 N Catalyst Reagent.

4.7 EDTA Calibration Standard.

5.0 Initial Power-Up Preparation (LECO FP 428)

5.1 Turn on the gas supplies and set the delivery pressures to 40 PSI.

5.2 Turn on the power switch.

5.3 Set the gas switch (off/standby/analyze) to the ANALYZE position.

5.4 Confirm that the pressure gauges on the front panel indicate:

5.4.1 Helium pressure - 12 PSI.

5.4.2 Back pressure - 5 PSI.

5.4.3 Combustion gas pressure - 1-2 PSI (If the pressures are incorrect they can be adjusted during system checkout.)

5.5 After a short delay the LECO logo will be displayed on the monitor.

5.6 Touch the globe on the logo to put in the log in mode.

5.7 For unsecured operation, press the USER icon and enter the operator's name.

5.8 Once the operator is logged in the Main Screen will be displayed.

5.9 At this point the System Checkout can be performed. If the System Checkout is not going to be done at this time, switch the gas switch to the STANDBY position.

6.0 System Checkout

6.1 All steps in the Initial Power-Up section must be completed first.

6.2 Switch the gas switch to the STANDBY position to conserve oxygen during the warm up period.

6.3 Allow the instrument to warm up for two hours.

6.4 Select the View option from the Main Screen and select the Submenu option from the pull-down menu.

6.5 Open the System Diagnostics folder by touching the icon and select Ambient Monitor.

6.6 The Ambient Monitor screen lists the actual temperature, voltages, amperage, and pressure readouts of the analog voltages that are read through the computer by the A/D converter. Nominal values and ranges are:

	Nominal Value	Range	
Ground Reference	0	0	Counts
5 Volt Reference	5.00	4.5 to 5.5	Volts
5 Volt Supply	5.00	4.5 to 5.5	Volts
+12 Volt Supply	12.00	10.8 to 13.2	Volts
-12 Volt Supply	-12.00	-10.8 to -13.2	Volts
+15 Volt Supply	15.00	13.5 to 16.5	Volts
-15 Volt Supply	-15.00	-13.5 to -16.5	Volts
24 Volt Supply	24.00	21.6 to 26.4	Volts
System Pressure	Variable	Variable	mm Hg
Reduction Heater	750	700 - 775	^{o}C
Furnace Temp.	950	25 - 1050	^{o}C
Cooler Temp.	5	2 - 10	^{o}C
TC Oven Temp.	45	43 - 47	^{o}C
TC Cell Current	90	85 - 95	mA
TC Cell Output	1.0	0.8 - 1.2	V

6.7 After reviewing the Ambient Monitor press the Escape key to return to the System Diagnostics folder.

6.8 Select the Leak Check icon and perform a Ballast Check, Oxygen Check and a Helium Check.

6.9 If a leak check is successfully completed after 60 seconds, an arrow will appear at the left of the bar graph in the "Good" region. If the leak check fails, the arrow will appear at the left of the bar graph in the "Leak" zone. If the leak check fails, the leak check should be reattempted. If it fails again, check the appropriate areas for leaks.

6.10 After completing the Leak Check procedure, press the Escape key until the system folders are displayed.

6.11 Open the System Configuration folder and select the Time and Date icon.

6.12 The time is set based on a 24 hour clock. Touch the Hours key until the correct hour appears. Repeat this procedure to set the minutes and seconds. When the correct time has been set, touch the Enter key.

6.13 The date is set in the same manner as the time. After the month, day, and year have been set, touch the Enter key. The display will return to the system folders.

6.14 Open the System Update folder and select the Barometer icon.

6.15 The system will wait for the pressure to stabilize and will then display the last barometric pressure that has been entered.

6.16 Set the barometric pressure by pressing the arrow keys to increase or decrease the pressure. When the current pressure is reached press the Enter key.

6.17 If necessary, adjust the gas pressure and flow as follows:

6.17.1 Helium Pressure and Flow. The helium pressure should read 12 PSI. If it does not, adjust with the helium pressure adjust on the front panel. Helium flow should be at the line on the rotameter (200 ml/min or standby flow of 30 ml/min). If necessary, adjust with the flow controller in the TC cell oven compartment which is accessible through the plugged hole in the oven compartment side panel.

6.17.2 Back Pressure. The back pressure should be 5 PSI. It can be adjusted with the 774-114 Pressure Regulator which is accessed by removing the left side panel of the instrument.

6.17.3 Combustion Gas (Oxygen) Pressure and Flow. The combustion gas pressure should indicate 1 - 2 PSI prior to analysis and 5 - 8 PSI during the analysis. This pressure is not adjustable. The flow is dependent on the oxygen profile set in the Analysis Constants procedure. If the reading goes above 10 PSI it indicates a blockage on the "oxygen" side of the system.

6.18 When all pressure settings are complete, return to the system folders screen and close the System Update folder. Then return to the Main Screen.

7.0 System Constants

7.1 Before a sample can be analyzed the System Constants must be programmed into the computer.

7.2 Select the View option from the Main Screen and select the Submenu option from the pull down menu.

7.3 Open the System Update folder and press the Analyze Const. icon. The table of constants appears. Check to see that each constant is at the nominal value.

Constant	Range	Nominal Value
Oxidation Furnace Temp	25 - 1050°C	850°C
Oxidation Furnace Standby Temp	25 - 900°C	600°C
Purge Cycles	0 - 30	3
Minimum Timeout	20 - 225 seconds	30 seconds
Comparator Level	0.01 - 100%	1.00
Calibration	0.0 - 10.0	Display Only
Blank (Protein)	0.0 - 100	Display Only
Protein Atm. Blank		0.000

7.4 If the Analyze Constants are set properly press the Escape key to bring back the open System Update folder.

7.5 Select the S.P.C. Const. icon.

7.6 The Protein Control Standard should be the expected nitrogen percentage in the standard used for standardization.

7.7 The upper and lower control limits should also be set.

7.8 When completed, press the Escape key to bring back the open System Update folder.

7.9 Select the Flow Profile icon and check to see that the table is set as follows:
Note: The Setting For # 2 Profile May Need To Be Adjusted To High 40.
To Compensate For Combustion Tube Flow Restriction.

#	O_2 Flow	Time
-1-	High	30
-2-	High	30
-3-	High	End

7.10 When completed, press the Escape key. This returns the open System Update folder. Close the open folder.

7.11 To return to the Main Screen, select Main at the top of the screen display.

8.0 Analysis of Standards and Samples

8.1 Set the OFF/STANDBY/ANALYZE switch to ANALYZE at least one hour before running standards and samples to allow the furnace temperature to reach the operating level.

8.2 EDTA standards, controls, and samples can be weighed in advance. The fineness of sample grind must be equivalent to that which gives a relative standard deviation (RSD) of $\leq$ 2.0% for 10 successive determinations of nitrogen in a 2 + 1 mixture of corn grain and soybeans that has been ground for analysis. This fineness is about 1.0 mm must be used for all mixed feeds and other nonhomogeneous materials. Weigh at least four separate 0.20mg portions of EDTA into individual foil capsules, fold properly, place in a labeled holder and record the weight to the nearest 0.1 mg. Nominal sample size is .200 - 250 mg if the volume doesn't exceed the capacity of the foil capsule. The maximum limit of nitrogen is 100 mg per sample. Weigh Samples (to the nearest 0.1 mg) into foil capsules, fold properly, and place in the holder. Weigh a duplicate sample for every 10 samples that are analyzed. Also weigh a control sample for every 10 samples that are analyzed plus 2 to 4 to run once calibration is complete.

8.3 To log in the samples, select Log In from the Main Screen.

8.4 Select Options on the Log In screen and select Pr Mode and As Rec as the options.

8.5 Press the ID's key and select the bank for EDTA.

8.6 Set the Edit: EDTA counter to 01.

8.7 Use the TAB key to get to the WEIGHT section. Type in the weight of the first EDTA standard and press ENTER.

8.8 Enter the weights of all of the EDTA standards in the same manner.

8.9 Press the ID's key and select the appropriate BANK for the control sample.

8.10 Set the counter back to 01 and enter the identification number and weights for the control samples to be run after calibration. Make sure the correct protein factor is entered.

8.11 Press ID's again and select the correct Bank for feed samples (class K).

8.12 Set the counter to 01 and enter sample log numbers and their corresponding weights. Make sure the correct protein factor is entered.

8.13 After every 10 samples press ID's and select the bank for duplicates.

8.14 Set the counter to 01 for the first duplicate and enter the duplicate log number and corresponding weight.

8.15 After the duplicate is entered, press ID's and select the bank for the control sample.

8.16 Set the counter to 01 for the first control sample and enter the identification number and corresponding weight.

8.17 When all the standards, samples, duplicates and controls have been logged in, press ID's and select the bank for blanks. Press Out Seq the number of times equivalent to the number of blanks to be run. Enough blanks must be run to reach a point of stability and reproducibility.

8.18 Then press ESCAPE to return to the Main Screen.

8.19 At this point it is advisable to check the ambient monitor. Select the Submenu option from the View menu.

8.20 Open the System Diagnostics folder and select Ambient Monitor. Compare the ambient monitor display to the table listed in the System Check Out section to verify that all parameters are stable and in the nominal range.

8.21 Escape from Ambient Monitor, close the folder and return to the Main Screen.

8.22 Select the Weight Stack from the view screen.

8.23 Press the Analyze icon and allow the blanks to run automatically. To halt the automatic run of the blanks, set the Manual/Auto switch to Manual just after the last blank has started to run.

8.24 Run blanks until the results are stable and reproducible.

8.25 If the average value of the reproducible blanks differs from 0.000 then do an update.

8.26 To update the blank, select System Folders from the View menu on the Main Screen.

8.27 Open the System Update folder and select Manual Blank. Update the current blank value by adding the average value of the reproducible blanks if it is negative or subtracting the average of the reproducible blanks if it is positive.

8.28 After the blank update has been done, run 2 or 3 blanks to make certain the proper adjustment was made.

8.29 Place the EDTA standards, control samples, feed samples, duplicates, etc. into the autosampler carousel in the order to be analyzed. A total of 19 samples can be loaded. Set the carousel in place on the instrument with position 20 over the combustion chamber entrance.

8.30 Set the Manual/Auto switch to Auto and press the Analyze icon on the Main Screen to begin the run. The results for the EDTA standards must become stable and reproducible.

8.31 If the average of the reproducible EDTA nitrogen results differs from the true value an update needs to be done.

8.32 First, stop the automated run by setting the Manual/Auto Switch to the Manual position just after the last EDTA standard drops into the combustion chamber.

8.33 Select System Folders from the View menu on the Main Screen.

8.34 Open the System Update folder and select Standard Calibration.

8.35 Type in the true nitrogen value for the EDTA standard and press ENTER. This will bring up the data base.

8.36 Select the reproducible EDTA runs and press Process Results. A new calibration factor will be calculated and displayed. Pressing Print will print the new factor.

8.37 Press Escape, close the folder and return to the Main screen.

8.38 Select the Weight Stack from the View menu. The Weight Stack will list the sequence in which automated sample analysis will be performed.

8.39 Switch the Manual/Auto switch to Auto and press the Analyze icon.

8.40 Check the results of the control sample to verify that they are within proper limits.

8.41 Fill the carousel as necessary during the run.

9.0 Maintenance

9.1 The nitrogen analyzer maintenance schedule will vary with individual applications.

9.2 The crucible replacement interval is typically every 50 analyses but will vary depending on sample material. To change:

9.2.1 Set the gas switch to the standby position and allow two minutes for system pressure to decrease.

9.2.2 Loosen the two thumbscrews holding the loading head.

9.2.3 Lift the loading head and set it off to one side. The lance will be extremely hot.

9.2.4 Screw the lance extractor tool into the hole on top of the lance head.

9.2.5 Remove the lance carefully and set it aside on a flame resistant material.

9.2.6 Using the crucible extractor tool, reach down into the combustion tube and grasp the crucible and lift it out and discard it in an appropriate container.

9.2.7 Place a new crucible in the combustion tube with the crucible extractor tool, replace the lance and remove the lance extractor tool.

9.2.8 Replace the loading head and tighten the two thumbscrews.

9.2.9 Wait at least 3 minutes for the system to reach operating temperature.

9.2.10 Run 2 - 4 blanks to purge air and moisture from the system.

9.3 The glass wool in the furnace filter tube should be replaced when approximately one-half of it is dirty.

9.4 Change the anhydrone and absorbent in the (right) measure flow reagent tube when the anhydrone becomes caked and/or the absorbent turns white.

9.5 Check the filter elements in the furnace filter and reagent tubes when the glass wool or reagents are replaced. Tap the filters lightly to remove any dust. If necessary, clean the filters in an ultrasonic cleaner.

9.6 Check the particle filter located after the furnace flow and before the thermoelectric cooler each month.

9.7 Check the honeycomb filter in the combustion tube monthly.

9.8 Periodically clean and inspect the furnace tube for pitting, cracks, or excessive wear. To clean the tube:

9.8.1 Shut off the gas supplies by switching the GAS key to the Off position.

9.8.2 Reset the furnace standby temperature to 25ºC in the Analysis Constants section of the System Update folder.

9.8.3 Remove the thumbscrews from the loading head assembly but do not disconnect the gas lines.

9.8.4 Remove the four screws that secure the sample drop block and remove the block.

9.8.5 Set aside the o-ring that seals the combustion tube.

9.8.6 Remove the screws that secure the cover plate and remove the plate.

9.8.7 Allow the combustion tube to cool to 25°C and then remove by lifting the tube straight upward.

9.8.8 Remove the crucible, aluminum oxide pellets, and the honeycomb filter, and then inspect the tube. If it is severely pitted or damaged, replace it; otherwise wash and dry the tube.

9.8.9 Replace the o-rings that seal the furnace tube every other time the furnace tube is repacked. Lightly grease the o-rings with silicone grease. Perform a leak check after the o-rings are replaced.

9.8.10 Repack the furnace tube according to the diagram in the instrument instruction manual.

9.8.11 Re-install the furnace tube.

9.8.12 Set the furnace temperature to the operating level. When the temperature has reached the operating level, perform oxygen and helium leak checks.

9.9 Change the anhydrone and absorbent in the (left) incoming helium reagent tube when the anhydrone becomes caked and/or the absorbent turns white. Check the filter in the tube when these reagents are replaced. Tap the filter lightly to remove any dust. If necessary, clean the filter in an ultrasonic cleaner.

9.10 Replace the disposable particle filters in the carrier and pneumatic flow lines every 6 - 12 months.

9.11 Replace the catalyst reagent in the catalyst heater tube when all of the copper sticks have turned black. To check the copper sticks, shut the furnace off and allow it to cool to room temperature. Then remove the tube and examine the copper sticks. If the catalyst reagent needs to be replaced, proceed as follows:

9.11.1 Shut off the gas supply.

9.11.2 Remove the top fitting of the catalyst tube by unscrewing the knurled bolt and then lift the catalyst tube up to remove it.

9.11.4 Repack the clean or new catalyst heater tube as shown in the instruction manual.

9.11.5 Reassemble the packed tube into the catalyst heater. Make sure the bottom end of the tube is positioned over the o-ring for a positive seal.

9.11.6 Replace the top fitting into the catalyst tube and secure it with a knurled bolt.

9.12 See the instruction manual for other maintenance procedures as needed.